In the Claims

Applicant has submitted a new complete claim set showing marked up claims with insertions indicated by underlining and deletions indicated by strikeouts and/or double bracketing.

Please cancel claims 58-80, 82-94, 96-109, 112-121, 123-145 and 147-161 without prejudice or disclaimer.

1. A method for labeling a target protein comprising

contacting a fusion protein with a biotin analog, and

allowing sufficient time for the biotin analog to be conjugated to the fusion protein via an acceptor peptide, in the presence of a biotin ligase mutant,

wherein the fusion protein is a fusion of the target protein and the acceptor peptide.

2. The method of claim 1, wherein the biotin analog comprises an aliphatic carboxylic acid tail.

3. The method of claim 1, wherein the biotin analog comprises a substitution at a trans-ureido nitrogen (N) of biotin.

4. The method of claim 1, wherein the biotin analog is selected from the group consisting of an N-ketone biotin analog, a ketone biotin analog, an N-azide biotin analog, an azide biotin analog, an N-acyl azide biotin analog, an NBD-GABA biotin analog, a 1,2-diamine biotin analog, an N-alkyne biotin analog and a tetrathiol biotin analog.

5. The method of claim 1, wherein the biotin analog is fluorogenic.

6. The method of claim 1, wherein the biotin analog is directly detectable.

7. The method of claim 6, wherein the biotin analog is coumarin, fluorescein, rhodamine, rosamine, an Alexa™ dye, resorufin, oregon green, tetramethyl rhodamine, Texas Red® or BODIPY.

8. The method of claim 1, wherein the biotin analog is labeled with a directly detectable label.

9. The method of claim 8, wherein the directly detectable label is selected from the group consisting of a fluorophore, a radioisotope, a contrast agent, an MRI contrast agent, a PET label, a phosphorescent label and a luminescent label.

10. The method of claim 1, wherein the biotin analog is labeled with an indirectly detectable label.

11. The method of claim 10, wherein the indirectly detectable label is selected from the group consisting of an enzyme, an enzyme substrate, an antibody, an antibody fragment, an antigen, a hapten, a ligand, an affinity molecule, a chromogenic substrate, a protein, a peptide, a nucleic acid, a carbohydrate and a lipid.

12. The method of claim 1, wherein the biotin analog is labeled with a membrane impermeant label.

13. The method of claim 1, wherein the biotin analog is labeled after conjugation to the fusion protein.

14. The method of claim 1, wherein the biotin analog is labeled with a singlet oxygen radical generator.

15. The method of claim 14, wherein the singlet oxygen generator is resorufin, malachite green, fluorescein or diaminobenzidine.

16. The method of claim 1, wherein the biotin analog is labeled with an analyte-binding group.

17. The method of claim 16, wherein the analyte-binding group is a metal chelator.

18. The method of claim 17, wherein the metal chelator is EDTA, EGTA, a pyridinium, an imidazole or a thiol.

19. The method of claim 1, wherein the biotin analog is labeled with a heavy atom carrier.

20. The method of claim 19, wherein the heavy atom carrier is iodine.

21. The method of claim 1, wherein the biotin analog is labeled with an affinity tag.

22. The method of claim 21, wherein the affinity tag is selected from the group consisting of a histidine tag, a GST tag, a FLAG tag and an HA tag.

23. The method of claim 1, wherein the biotin analog is labeled with a photoactivatable cross-linker.

24. The method of claim 23, wherein the photoactivatable cross-linker is selected from the group consisting of benzophenones and aziridines.

25. The method of claim 1, wherein the biotin analog is labeled with a photoswitch label.

26. The method of claim 25, wherein the photoswitch label is an azobenzene.

27. The method of claim 1, wherein the biotin analog is labeled with a photolabile protecting group.

28. The method of claim 27, wherein the photolabile protecting group is a nitrobenzyl group, a dimethoxy nitrobenzyl group or NVOC.

29. The method of claim 1, wherein the biotin analog is labeled with a peptide comprising non-naturally occurring amino acids.

30. The method of claim 1, wherein the target protein is a cell surface protein.

31. The method of claim 1, wherein the fusion protein is in a cell.

32. The method of claim 31, wherein the cell expresses the biotin ligase mutant.

33. The method of claim 31, wherein the cell is a eukaryotic cell.

34. The method of claim 31, wherein the cell is a bacterial cell.

35. The method of claim 33, wherein the eukaryotic cell is a mammalian cell, a Drosophila cell, a Zebrafish cell, a Xenopus cell, a yeast cell or a C. elegans cell.

36. The method of claim 1, wherein the acceptor peptide comprises an amino acid sequence of SEQ ID NO: 4.

37. The method of claim 1, wherein the acceptor peptide comprises an amino acid sequence of SEQ ID NO: 5.

38. The method of claim 1, wherein the acceptor peptide is N- or C- terminally fused to the target protein.

39. The method of claim 1, wherein the biotin ligase mutant has an amino acid substitution at 83, 89, 90, 91, 92, 107, 112, 115, 116, 117, 118, 123, 132, 134, 142, 186, 188, 189, 190, 204, 206, 207 and/or 235.

40. The method of claim 39, wherein the amino acid substitution is at T90, C107, Q112, G115, Y132, S134, V189 and/or I207.

41. The method of claim 40, wherein the amino acid substitution is at T90.

42. The method of claim 41, wherein the amino acid substitution is selected from the group consisting of T90G, T90A and T90V.

43. The method of claim 42, wherein the amino acid substitution is T90G.

44. The method of claim 43, wherein the biotin analog is N-ketone biotin analog.

45. The method of claim 43, wherein the biotin ligase mutant has an amino acid sequence of SEQ ID NO: 6.

46. The method of claim 41, wherein the biotin ligase mutant further comprises an amino acid substitution at N91.

47. The method of claim 46, wherein the amino acid substitution at N91 is N91S, N91G, N91A or N91L.

48. The method of claim 47, wherein the biotin ligase mutant comprises amino acid substitutions of T90G and N91S.

49. The method of claim 48, wherein the biotin analog is N-alkyne biotin analog.

50. The method of claim 48, wherein the biotin ligase mutant has an amino acid sequence of SEQ ID NO: 7.

51. The method of claim 1, wherein the biotin ligase mutant comprises amino acid substitutions of T90G/N91G, T90A/N91A or T90A/N91L.

52. The method of claim 39, wherein the amino acid substitution is C107G, Q112M, G115A, Y132G, Y132A, S134G, V189G and/or I207S.

53. The method of claim 1, wherein the method is performed in a cell free environment.

54. The method of claim 1, wherein the method is performed in a cell.

55. The method of claim 1, wherein the method is performed in a subject.

56. The method of claim 1, wherein the acceptor peptide is fused to the target protein via a cleavable bond or linker.

57. A composition comprising
a biotin ligase mutant that binds to a biotin analog.

58.-80. (Canceled)

81. A composition comprising
a nucleic acid encoding a biotin ligase mutant comprising an amino acid substitution at 83, 89, 90, 91, 92, 107, 112, 115, 116, 117, 118, 123, 132, 134, 142, 186, 188, 189, 190, 204, 206, 207 and/or 235.

82.-94. (Canceled)

95. A composition comprising
a biotin analog that binds to a biotin ligase mutant,
wherein the biotin analog is alkylated at a trans-ureido nitrogen (N) of biotin.

96.-109. (Canceled)

110. A composition comprising

a biotin analog that binds to a biotin ligase mutant,

wherein the biotin analog is ketone biotin analog or NBD-GABA.

111. A phage display library comprising a biotin ligase mutant having an amino acid substitution at 83, 89, 90, 91, 92, 107, 112, 115, 116, 117, 118, 123, 132, 134, 142, 186, 188, 189, 190, 204, 206, 207 or 235.

112.-121. (Canceled)

122. A method for identifying a biotin ligase mutant having specificity for a biotin analog comprising

contacting a biotin analog with an acceptor peptide in the presence of a candidate biotin ligase mutant molecule, and

detecting a biotin analog that is bound to the acceptor peptide,

wherein the presence of a biotin analog bound to an acceptor peptide indicates that the candidate biotin ligase mutant molecule is a biotin ligase mutant having specificity for a biotin analog.

123.-145. (Canceled)

146. A method for identifying a biotin analog having specificity for a biotin ligase mutant comprising

combining an acceptor peptide with a labeled biotin in the presence of a biotin ligase mutant and determining a control level of biotin incorporation,

combining an acceptor peptide with a labeled biotin and a candidate biotin analog molecule in the presence of a biotin ligase mutant and determining a test level of biotin incorporation, and

comparing the control and test levels of biotin incorporation,

wherein a test level that is less than a control level is indicative of a biotin analog having specificity for a biotin ligase mutant.

147.-161. (Canceled)